Sample Preparation of Cannabis and Cannabis Products for Heavy Metals Analysis

1.0 Scope and Application

1.1 This method was adapted from the Selected Analytical Methods for Environmental Remediation and Recovery (SAM), Method 3050B: Acid Digestion of Sediments, Sludges, and Soils, published by the U.S. Environmental Protection Agency (EPA) and the Elemental Analysis Manual (EAM) for Food and Related Products published by the U.S. Food and Drug Administration (FDA).

1.2 This standard operating procedure (SOP) describes two acid digestion methods for the preparation of cannabis and cannabis products samples for the analysis of samples by inductively coupled plasma mass spectrometry (ICP-MS).

1.3 The first method describes acid digestion without microwave assistance, a method that IS NOT a total digestion technique for most samples. The second method describes microwave assisted acid digestion to determine total-extractable heavy metals concentration in samples.

1.4 The first method will dissolve almost all elements that could become "environmentally available." By design, elements found in silicate structures are not normally dissolved by the second procedure as they are not usually mobile in the environment. If total digestion is required, then use the second method.

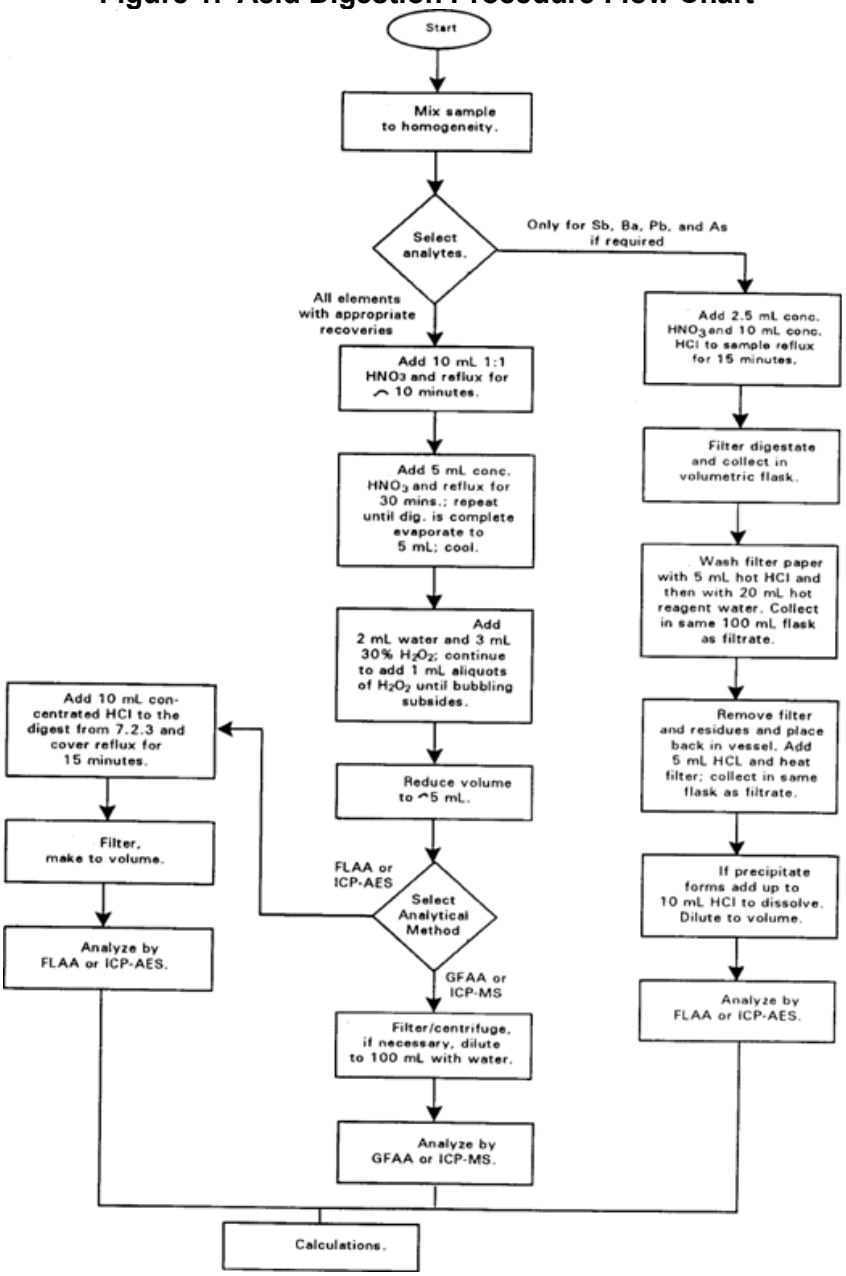
1.5 Other matrices may be analyzed by these procedures if performance is verified in the matrix of interest, at the concentration levels of interest. These methods should only be used by analysts familiar with trace element analysis and ICP-MS. The analyst must be trained in the interpretation of spectral and matrix interferences and procedures for their correction.

2.0 Summary of the Method

2.1 Acid Digestion

2.1.1 For the digestion of samples, a representative 0.5-2g sample is digested with repeated additions of nitric acid (HNO_3) and hydrogen peroxide (H_2O_2). For ICP-MS analysis, the resultant digestate is reduced in volume while heating and then diluted to a final volume of 100mL. If required, a separate sample aliquot shall be dried for a total percent solids determination. Figure 1 shows the method procedure.

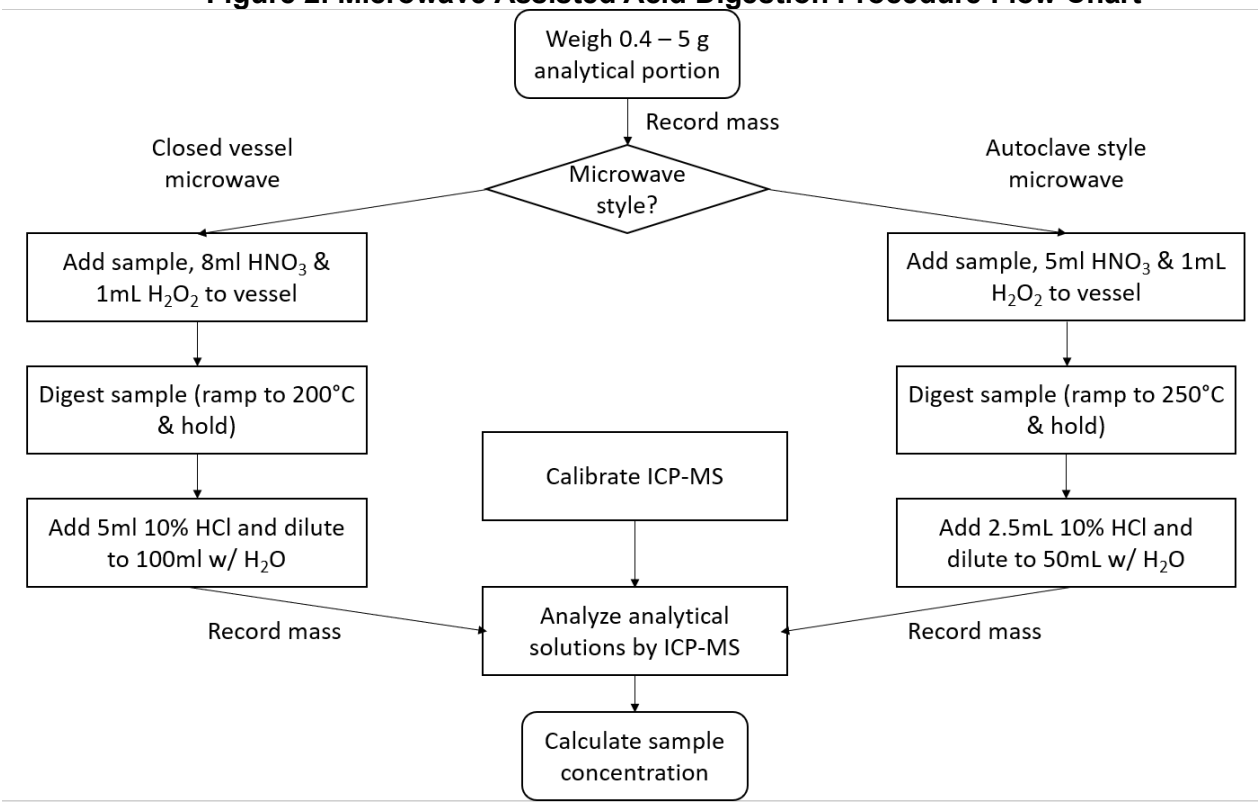
Figure 1. Acid Digestion Procedure Flow Chart



2.2 Microwave Assisted Acid Digestion

2.2.1 An analytical portion of cannabis or cannabis product is decomposed in acid inside a high-pressure digestion vessel using microwave heating.[1, 2] The analytical solution is analyzed using an inductively coupled plasma mass spectrometer (ICP-MS). Elemental concentrations are quantified using external calibration and quality controls are incorporated to ensure data quality. Figure 2 shows the method procedure.

Figure 2. Microwave Assisted Acid Digestion Procedure Flow Chart



2.3 ICP-MS Instrument Analytical Limits

2.3.1 Typical analytical limits are listed in Table 1 but will vary depending on the specific instrumentation, dilution factor and blank quality. Significantly lower limit of detection (LOD) and limit of quantitation (LOQ) have been achieved for several target analytes for different matrices and larger sample masses. Achieving the lowest limits requires meticulous attention to operating conditions and the highest level of quality control for each set of analyses.

Table 1. Nominal Analytical Limits for Multi-Lab Validated Elements

Element	Symbol	MBK_L (µg/kg)	MBK_C (µg/kg)	ASDL [a] (µg/kg)	ASQL [a] (µg/kg)	LOD [b] (µg/kg)	LOQ [b] (µg/kg)
Arsenic	^{75}As	0.00340	0.0111	0.0127	0.116	1.27	11.6
Cadmium	^{111}Cd	0.000635	0.00311	0.00408	0.0371	0.408	3.71
Mercury	^{201}Hg	0.00171	0.00693	0.00861	0.0782	0.861	7.82
Lead	^{sum}Pb	0.00455	0.0118	0.0120	0.109	1.20	10.9

[a.] Based upon method blanks measured during the single lab validation over 1 year; n = 143

[b.] Based upon 0.5 g analytical portion and 50 g analytical solution (DF ≈ 100x)

3.0 Interferences

3.1 Cannabis samples can contain diverse matrix types, each of which may present its own analytical challenge. Spiked samples and any relevant standard reference

material should be processed in accordance with the quality control requirements given in Sec. 7.0.

4.0 Equipment and Supplies

Disclaimer: The use of trade names in this method constitutes neither endorsement nor recommendation by the Washington State Department of Agriculture. Equivalent performance may be achievable using apparatus and materials other than those cited here.

4.1 Acid Digestion (*Method 1*)

4.1.1 Digestion Vessels - 250-mL.

4.1.2 Vapor recovery device (e.g., ribbed watch glasses, appropriate refluxing device, appropriate solvent handling system).

4.1.3 Drying ovens - able to maintain 30°C ± 4°C.

4.1.4 Temperature measurement device capable of measuring to at least 125°C with suitable precision and accuracy (e.g., thermometer, IR sensor, thermocouple, thermistor, etc.)

4.1.5 Filter paper - Whatman No. 41 or equivalent.

4.1.6 Centrifuge and centrifuge tubes.

4.1.7 Analytical balance - capable of accurate weighings to 0.01 g.

4.1.8 Heating source - Adjustable and able to maintain a temperature of 90-95°C. (e.g., hot plate, block digestor, microwave, etc.)

4.1.9 Funnel or equivalent.

4.1.10 Graduated cylinder or equivalent volume measuring device.

4.1.11 Volumetric Flasks - 100-mL.

4.2 Microwave Assisted Acid Digestion (*Method 2*)

4.2.1 Inductively coupled plasma mass spectrometer (ICP-MS)—Capable of scanning mass-to-charge (m/z) range 5 – 240 amu with a minimum resolution of 0.9 amu at 10% peak height. Must have collision/reaction cell that can be pressurized with helium and kinetic energy discrimination for polyatomic interference attenuation. Method was developed on Agilent™ models 7500ce, 7700x, 7900 and 8800 and directions are specific to Agilent brand equipment. Use of the method with other brands of instruments or models may require procedural modifications.

4.2.2 Microwave digestion system—Requires temperature control to at least 200 °C and pressures ≥ 300 psi (~ 20 bar) with appropriate safety features to prevent over-pressurization of vessels. Microwave must have multi-step programming with ramp to temperature capability. Digestion vessels must be PFA, TFM Teflon® lined or quartz. Directions on use of microwave digestion equipment are specific to CEM™ or Milestone™. Method was developed using CEM MARS Xpress™ and Milestone UltraWAVE™ and UltraCLAVE™ III systems.

4.2.3 Labware—All laboratory ware must be sufficiently clean for trace metals analysis. The recommended cleaning procedure for all laboratory ware includes washing

with clean rinsing laboratory detergent such as Micro-90, reagent water rinse, soaking in 10% nitric acid and final reagent water rinse. Glass should not be used because of possible contamination. Labware can be tested for contamination before using a particular lot with 1% nitric acid. Virgin (non-recycled) Teflon® FEP, PFA, PP, LDPE or HDPE are recommended materials. Non-metal spatulas should be used for sampling cannabis portions.

4.2.4 Gloves—Use powder free vinyl or nitrile. Do not use powdered or latex gloves because of possible contamination. Gloves intended for clean rooms and are free from metals contamination are suggested.

4.2.5 Analytical balance—Capable of measuring to 0.1 mg.

4.2.6 Top Loading balance—Capable of measuring to 0.01 g.

4.2.7 Micropipettes—Air displacement micropipettes with metal free colorless disposable plastic tips. Do not use colored tips due to possible contamination. If applicable, remove metal tip ejector to avoid potential contamination.

4.2.8 Clean air hood/canopy—Class 100 polypropylene metal free hoods/canopies are recommended for sample handling.

4.2.9 Peristaltic pump tubing—Recommended sample and internal standard (ISTD) peristaltic pump tubing is black:black (0.76 mm inner diameter). At 0.1 rev/s (6 RPM) approximately 200 µL/min sample and 200 µL/min ISTD are delivered to the nebulizer (see Figure 2).

4.2.9.1 The 1:1 sample-to-ISTD ratio dilutes the sample 2x inside the mixing tee so that digests can be diluted to 50 g directly into an autosampler vial.

4.2.9.2 A 1:1 sample-to-ISTD ratio also ensures both sample and ISTD pump tubing stretch at the same rate over and reduces instrumental drift.

4.2.10 A ~16:1 sample-to-ISTD ratio has been previously used in “Draft Method for Analysis of Foods for As, Cd, Cr, Hg and Pb by ICP-MS CFSAN/ORS/DBC /CHCB April 25, 2011.” Other pump tubing sizes are acceptable, <u>but all QCs must pass</u> to show adequate performance.

4.2.11 Notes

4.2.11.1 This method was validated with 1:1 sample to ISTD mixing. Solution concentrations and matrices listed assume 1:1 sample-to-ISTD.

4.2.11.2 For 16:1 sample-to-ISTD ratio (Agilent default), the sample pump tubing is white:white (1.02 mm i.d.) and ISTD is orange:blue (0.25 mm i.d.).

4.2.11.3 If opting for sample-to-ISTD other than 1:1, the following adjustments must be made:

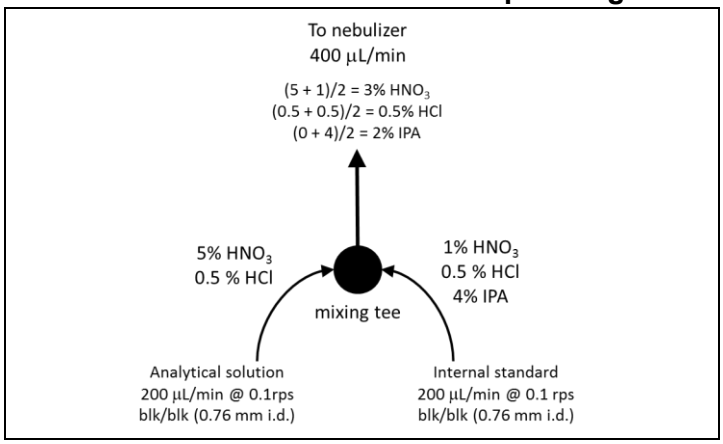
4.2.11.3.1 Assume 50% acid consumption during digestion and matrix match standards to analytical solutions.

4.2.11.3.2 Make corresponding adjustments in ISTD and isopropanol concentrations.

4.2.12 Drain tubing

4.2.12.1 Recommended drain tubing is yellow:blue (1.52 mm i.d.) or larger which drains > 650 µL/min from the spray chamber. Smaller drain tubing will cause spray chamber flooding.

Figure 3. Recommended Peristaltic Pump Tubing Schematic



5.0 Reagents and Standards

Always use high purity or trace metals grade reagents. Blank levels will be <LOQ if using best laboratory practices and high purity reagents.

Safety Notes: Reagents should be regarded as potential health hazards and exposure to these materials should be minimized. Follow universal precautions. Wear gloves, a lab coat, and safety glasses while handling reagents.

Exercise caution when handling and dispensing concentrated acids. Always add acid to water. Acids are caustic chemicals that are capable of causing severe eye and skin damage. If acids or bases come in contact with any part of the body, quickly wash the affected area with copious quantities of water for at least 15 minutes.

5.1 Acid Digestion (*Method 1*)

5.1.1 Reagents

5.1.1.1 Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination. If the purity of a reagent is questionable, analyze the reagent to determine the level of impurities. The reagent blank must be less than the MDL to be used.

5.1.1.2 Reagent Water. Reagent water will be interference free. All references to water in the method refer to reagent water unless otherwise specified. Refer to Chapter One for a definition of reagent water.

5.1.1.3 Nitric acid (concentrated), HNO_3. Acid should be analyzed to determine level of impurities. If method blank is < MDL, the acid can be used.

5.1.2 Hydrochloric acid (concentrated), HCl. Acid should be analyzed to determine level of impurities. If method blank is < MDL, the acid can be used.

5.1.3 Hydrogen peroxide (30%), H_2O_2. Oxidant should be analyzed to determine level of impurities. If method blank is < MDL, the peroxide can be used.

5.2 Microwave Assisted Acid Digestion (*Method 2*)

5.2.1 Reagents

5.2.1.1 Reagent water—Water meeting specifications for ASTM Type-I water[3].

5.2.1.2 Argon supply—High purity (99.99%) argon.

5.2.1.3 Helium for collision cell—Ultra high purity (99.999%)

5.2.1.4 High purity nitric acid—Concentrated (67-70%, sp. Gr. 1.42), double distilled. The trade name for double distilled grade will vary by manufacturer.

5.2.1.5 High purity hydrochloric acid—Concentrated (30-35%, sp. Gr. 1.18), double distilled.

5.2.1.6 High purity isopropanol—Electronic grade or equivalent.

5.2.1.7 Nitric acid (for cleaning)—Concentrated (sp gr 1.42), trace metals grade.

5.2.1.8 Hydrogen Peroxide—Concentrated (30%), high purity or trace metals grade.

5.2.2 Solutions

5.2.2.1 Hydrochloric acid (HCl) 10% (v/v)—Dilute 200 mL (236 g) high purity HCl to 2,000 mL with reagent water.

Recommendation: Prepare solution in an empty bottle originally used for concentrated hydrochloric acid. Dilute gravimetrically on a top loading balance with a capacity of a least 2500 g. Tare bottle. Fill with approximately 1000 mL reagent water. Note mass. Add approximately 200 g acid while pouring slowly from the stock bottle. Add the remaining acid from a Teflon squeeze bottle to enable fine control of acid addition. The total mass of concentrated hydrochloric acid added should be 236 g (200 mL * 1.18 g/mL = 236 g). Add reagent water until a total solution mass of ~2036 g is reached (1800 g water + 236 g HCl). Cap bottle and mix.

5.2.2.2 Diluent and rinse solution 5% Nitric Acid (HNO_3) & 0.5% HCl (v/v)—Dilute 100 mL HNO_3 (142 g) and 10 mL (11.8 g) HCl to 2,000 mL with reagent water.

Recommendation: Use an empty bottle originally used for concentrated hydrochloric or nitric acid. Dilute gravimetrically on a top loading balance with a capacity of a least 2500 g if making 2L of solution. Tare bottle. Fill with

approximately 1000 g reagent water. Note mass. Add 11.8 g (10 mL) high purity HCl (double distilled, 30-35%). Swirl to mix. Add 142 g (100 mL) high purity HNO_3 (double distilled, 67-70%). Dilute with reagent water to 2L or ~2044 g. It is recommended to add concentrated acids either with a high purity bottle top acid dispenser or Teflon PFA squeeze bottle.

5.2.2.3 Internal standard solution (ISTD)—Multi-element solution prepared by diluting an appropriate volume of stock standard. ISTD matrix is 1% HNO_3, 0.5% HCl and 4% isopropanol. The presence of isopropanol will help equalize arsenic and selenium sensitivities due to residual carbon post digestion.[4] The ISTD dilution factor is 1:1 if the autosampler and internal standard peristaltic pump tubes are equal inner diameter. The analytical solution pumped into the nebulizer will be approximately 2% isopropanol.

5.2.2.3.1 ISTD solution may be prepared volumetrically. The exact concentration is not as important as maintaining the same concentration over an analytical sequence.

5.2.2.3.2 ISTD elements and suggested concentrations: 20 ng Ge/g, 2.5 ng Rh/g, 5 ng Ir/g, and 2.5 ng Bi/g. These concentrations are only suggestions. Labs may alter at their discretion if QC passes.

5.2.2.3.3 0.1% (v/v) Triton X-100 added to the ISTD may help stabilize signal. Triton X100 is permitted but labs must ensure that LOD and LOQs are also established with this ISTD mixture, and all QC must pass.

5.2.2.3.4 If using default Agilent tubing (1.02 mm i.d. sample white/white and 0.25 mm i.d. orange/blue) then increase IPA and ISTD elemental concentrations approximately 8x. The exact concentration is not important.

5.2.2.4 Recommended Tuning Solution—2 µg/L Li, Co, Y, Ce, and Tl solution in 5% HNO_3 – 0.5% HCl and 4% isopropanol used to tune ICP-MS.
The method suggests sample tubing and ISTD tubing to be equal diameter, diluting tune solution by 2×. Therefore, tune solution should be 2 µg/L so that 1 µg/L is aspirated into the ICP (see 4.2 Figure 3).

5.2.3 Calibration Standard Solutions

5.2.3.1 Analyte stock standard solutions—Commercially prepared single element traceable standard solutions in acid matrices prepared specifically for plasma mass spectrometric analysis should be used. Custom made multi-element solutions may be economically viable when one considers the time savings they provide.

5.2.3.1.1 Standards can be purchased on a mass/mass basis to eliminate density correction factors. If standards are mass/volume, then perform a density correction.

5.2.3.1.2 Use standard solutions prior to expiration. Solutions may slowly become more concentrated due to loss of water vapor through the bottle material and evaporation while the bottle is uncapped.

5.2.3.1.3 Example of a multi-element custom standard:

Hg: 1 µg/g

As, Cr, Cd, Ni, Mo, Se, Tl, Pb: 10 µg/g

Mn: 50 µg/g

Cu, Zn: 100 µg/g

5.2.3.2 Intermediate standard solutions—Dilute stock standards with 5% HNO_3 – 0.5% HCl diluent. Store in Teflon® FEP, PP or HDPE bottles. Single element standards may be combined in the same solution to prepare multi-element calibration standard solutions.

5.2.3.2.1 All calibration standards shall be prepared on a mass/mass basis. Standard certificates of analyses often provide density information.

5.2.3.2.2 We recommend making an intermediate standard by gravimetrically diluting the multielement custom standard mix by 100x.

5.2.3.3 Standard solutions—Dilute intermediate standard with 5% HNO_3 – 0.5% HCl to prepare multi-element working standards. Store in Teflon® FEP, PP or HDPE bottles.

5.2.3.3.1 Hg concentration should be kept low to minimize memory effects (carryover) and wash out times. **The high standard (level 5 in table 2) is optional**, but it is needed if analytical solution concentrations are higher than the level 4 standard. Preferably, the analyst may further dilute the sample so that the analytical solution concentration falls within the calibration range. Longer washout times may be needed if there is Hg carryover. An Au rinse (~1 ppm Au) after samples containing high Hg may be added to assist washout.

5.2.3.3.2 High concentrations of Mn, Cu and Zn are often present in plants compared to As, Cr, Cd, Tl, Pb, Ni, Mo, Se and Hg. Some plants are fortified in Se and will have high Se levels.

5.2.3.3.3 Table 2 is an example of standard concentrations. These may be changed if QC passes, and analytical solution concentrations fall within the calibration range.

Table 2. Example of calibration curve standard concentrations

Analyte	Level 1 (ng/g)	Level 2 (ng/g)	Level 3 (ng/g)	Level 4 (ng/g)	*Level 5 (ng/g) Optional*
Hg	0	0.01*	0.1	1.0	*2.5*
As	0	0.1	1.0	10.0	*25.0*
Cd	0	0.1	1.0	10.0	*25.0*
Pb	0	0.1	1.0	10.0	*25.0*

* This level Hg may be less than instrument LOD.

5.2.3.4 Standard blank—5% HNO_3–0.5% HCl.

5.2.3.5 Initial calibration verification (ICV)—Dilute an appropriate volume of stock ICV solution gravimetrically with 5% HNO_3–0.5% HCl so analyte concentration will be at the approximate midpoint of the calibration curve. ICV and calibration standard solutions should be prepared from different stock solutions (second source).

5.2.3.6 Continuing calibration verification (CCV)—Use a mid-level standard.

6.0 Digestion Procedure

6.1 Acid Digestion (*Method 1*)

6.1.1 Mix the sample thoroughly to achieve homogeneity. All equipment used for homogenization should be cleaned to minimize the potential of cross-contamination. For each digestion procedure, weigh to the nearest 0.01 g and transfer a 0.5-2 g sample to a digestion vessel. For samples with high liquid content, a larger sample size may be used if digestion is completed.

6.1.1.1 NOTE: All steps requiring the use of acids should be conducted under a fume hood by properly trained personnel using appropriate laboratory safety equipment. The use of an acid vapor scrubber system for waste minimization is encouraged.

6.1.2 For the digestion of samples for analysis by ICP-MS, add 10 mL of 1:1 HNO3, mix the slurry, and cover with a watch glass or vapor recovery device. Heat the sample to 95°C ± 5°C and reflux for 10 to 15 minutes without boiling. Allow the sample to cool, add 5 mL of concentrated HNO3, replace the cover, and reflux for 30 minutes. If brown fumes are generated, indicating oxidation of the sample by HNO3, repeat this step (addition of 5 mL of conc. HNO3) over and over until no brown fumes are given off by the sample indicating the complete reaction with HNO3. Using a ribbed watch glass or vapor recovery system, either allow the solution to evaporate to approximately 5 mL without boiling or heat at 95°C ± 5°C without boiling for two hours. Always maintain a covering of solution over the bottom of the vessel.

6.1.2.1 NOTE: Alternatively, for direct energy coupling devices, such as a microwave, digest samples for analysis ICP-MS by adding 10 mL of 1:1 HNO3, mixing the slurry and then covering with a vapor recovery device. Heat the sample to 95°C ± 5°C and reflux for 5 minutes at 95°C ± 5°C without boiling. Allow the sample to cool for 5 minutes, add 5 mL of concentrated HNO_3, heat the sample to 95°C ± 5°C and reflux for 5 minutes at 95°C ± 5°C. If brown fumes are generated, indicating oxidation of the sample by HNO3, repeat this step (addition of 5 mL concentrated HNO_3) until no brown fumes are given off by the sample indicating the complete reaction with HNO_3. Using a vapor recovery system, heat the sample to 95°C ± 5°C and reflux for 10 minutes at 95°C ± 5°C without boiling.

6.1.3 After the previous steps have been completed and the sample has cooled, add 2 mL of water and 3 mL of 30% H_2O_2. Cover the vessel with a watch glass or vapor recovery device and return the covered vessel to the heat source for warming and to start the peroxide reaction. Care must be taken to ensure that

losses do not occur due to excessively vigorous effervescence. Heat until effervescence subsides and cool the vessel.

6.1.3.1 NOTE: Alternatively, for direct energy coupled devices: After the 6.1.2.1 "NOTE" step has been completed and the sample has cooled for 5 minutes, add slowly 10 mL of 30% H_2O_2. Care must be taken to ensure that losses do not occur due to excessive vigorous effervescence. Go to 6.1.5.

6.1.4 Continue to add 30% H_2O_2 in 1-mL aliquots with warming until the effervescence is minimal or until the general sample appearance is unchanged.

6.1.4.1 NOTE: Do not add more than a total of 10 mL 30% H_2O_2.

6.1.5 Cover the sample with a ribbed watch glass or vapor recovery device and continue heating the acid-peroxide digestate until the volume has been reduced to approximately 5 mL or heat at 95°C ± 5°C without boiling for two hours. Always maintain a covering of solution over the bottom of the vessel.

6.1.5.1 NOTE: Alternatively, for direct energy coupled devices: Heat the acid-peroxide digestate to 95°C ± 5°C in 6 minutes and remain at 95°C ± 5°C without boiling for 10 minutes.

6.1.6 After cooling, dilute to 100 mL with water. Particulates in the digestate should then be removed by filtration, by centrifugation, or by allowing the sample to settle. The sample is now ready for analysis by ICP-MS.

6.1.6.1 Filtration - Filter through Whatman No. 41 filter paper (or equivalent).

6.1.6.2 Centrifugation - Centrifugation at 2,000-3,000 rpm for 10 minutes is usually sufficient to clear the supernatant.

6.1.7 The diluted digestate solution contains approximately 5% (v/v) HNO_3. For analysis, withdraw aliquots of appropriate volume and add any required reagent or matrix modifier.

6.1.8 Calculations:

6.1.8.1 The concentrations determined are to be reported based on the actual weight of the sample.

6.2 Microwave Assisted Acid Digestion (*Method 2*)

6.2.1 Terms and definitions:

6.2.1.1 A "digestion batch" is defined as digests from a single rotor undergoing the same digestion program at the same time. For example, a CEM MARS Xpress digestion batch will have up to 40 vessels in a digestion batch.

6.2.1.2 An "analytical sequence" is comprised of the analytical solutions analyzed during a single sequence following instrument tuning and optimization and with one calibration. An analytical sequence may contain solutions from more than one digestion batch.

Perform the following operations in a clean environment to reduce contamination. Use an exhausting hood when working with nitric acid. Potential elements of interest (e.g. Cr, Ni, Mo, Co and Fe) may leach from stainless steel and contaminate plants, especially when they are acidic or

tough to grind. Care should be taken to prevent leaching of these elements during contact with metallic (stainless steel) equipment. Replace stainless steel grinding components with titanium or tungsten carbide when possible. Cryogrinding is often useful for sticky and gummy matrices that do not grind well in a typical knife mill blender.

Considerations of acid concentration in analytical solutions: Nitric acid is lost or consumed during digestion by reaction with an organic sample, high temperature decomposition and venting as a vapor. HCl is added immediately after digestion to stabilize Hg.[2] Assuming 50% acid consumption during digestion, the final matrix concentration is 4-5% HNO_3 and 0.5% HCl. The ISTD matrix is 1% HNO_3 and 0.5% HCl. Analytical solution and ISTD are combined in a 1:1 ratio inside a mixing tee so that the final matrix aspirated into the spray chamber is approximately 3% HNO_3 and 0.5% HCl with 2% isopropanol (see Figure 2). Mixing internal standard at a 1:1 ratio narrows the range of acid concentrations and total dissolved solids introduced into the plasma.

6.2.2 Digestion Procedure Using Conventional Closed or Self-Venting Vessels

6.2.2.1 Add a few drops of reagent grade deionized water to each vessel prior to taring to prewet the analytical portion.

6.2.2.2 A minimum of 2 MBKs must be included in each digestion batch to verify the absence of contamination that may arise from the vessels. Place MBKs in random vessels.

6.2.2.3 Weigh analytical portion into clean vessel liner and record analytical portion mass to the nearest 0.1 mg.

6.2.2.3.1 For samples of unknown composition limit the dry-mass equivalent of product to no more than 0.5 g. If maximum pressure attained for this unknown is less than the vessel limit, then a greater mass may be analyzed.

6.2.2.3.2 Use less than 0.5 g for samples high in salt or fat.

6.2.2.3.3 Use an analytical portion mass of 5 g for liquids. An analytical portion of 5 g should not be exceeded even if calculations indicate that a larger portion could be taken.

6.2.2.3.4 Use 1 g reagent water for method blanks (MBK) and optional fortified method blanks (FMB).

6.2.2.3.5 Add 1 g of reagent water with dry plant and CRMs to help control exothermic reactions.

6.2.2.4 Add 8.0 mL of high purity nitric acid (11.3 g) to vessel liner, washing down any material on walls. A bottle top acid dispenser is suggested. Acid should be added drop wise until it can be established that the sample will not react violently. If foaming or reaction with the acid is observed, let the vessels sit uncovered in a clean hood until reaction subsides. If a clean hood is unavailable, place caps on vessels without pressing down fully or, if so equipped, cap vessels but loosen the pressure relief nut (with the safety membrane) to allow pressure to escape. If, however, it appears that

excessive foaming would result in the sample-acid mixture expanding out of the vessel then cap the vessel and tighten to appropriate torque to prevent loss of sample or acid.

6.2.2.5 Add 1 mL high purity 30% H_2O_2 to each vessel. It may be necessary to pre-digest for more than 20 minutes before adding H_2O_2 if samples foam excessively.

6.2.2.6 2 mL H_2O_2 may further eliminate excess dissolved carbon and reduce its corresponding interference ArC^+ at m/z 52 and matrix effects. If using 2 mL H_2O_2, ensure LOD/LOQ do not change.

6.2.2.7 Seal vessels, apply correct torque to cap (tighten pressure relief nuts if equipped) and run the digestion program in Table 3.

Table 3. Closed vessel style microwave digestion program

Digestion Programs for CEM MARS XPress™ with 40-Position Carousel with Ramp to Temperature Feature

Power is applied for the Ramp Time minutes or until Control Pressure or Control Temperature is met. If Control Pressure or Control Temperature are met before end of Ramp Time, then program proceeds to Hold Time

	Digestion
Maximum Power	100% (1600W[a])
Ramp Time (min)	25
Hold Time (min)	15
Control Temperature (°C)	200

[a]Other power level microwaves are acceptable

6.2.2.8 Two cycle microwave digestion programs are allowed, but LOD/LOQ must not change, and all QC must pass.

6.2.2.9 After vessels have cooled to less than 50°C move to an exhausting clean hood and vent excess pressure slowly. Quantitatively transfer digests to a clean container and dilute digestion solution to approximately 50 g with reagent water followed by 0.5 mL (1.2 g) high purity HCl (or 5 mL of 10% HCl solution). Add more reagent water for a final volume of 100 mL and record final analytical solution mass. The mass of a 100 mL 5% HNO_3 – 0.5% HCl analytical solution is approximately 102 g.

Note: Gravimetric dilution is recommended into a trace element free 100 mL polyethylene or polypropylene bottle. Total dilution factor will be approximately 200 (0.5 g analytical portion to 102 g analytical solution).

6.2.3 Digestion Procedure Using Microwave Autoclave Style Digestion Systems

6.2.3.1 Add a few drops of reagent grade deionized water to each vessel prior to taring to prewet the analytical portion.

6.2.3.2 A minimum of 2 MBKs must be included in each digestion batch to verify the absence of contamination that may arise from the vessels. Place MBKs in random vessels.

6.2.3.3 Weigh analytical portion into clean vessel liner and record analytical portion mass to the nearest 0.1 mg.

6.2.3.3.1 For samples of unknown composition limit the dry-mass equivalent of cannabis to no more than 0.5 g.

6.2.3.3.2 Use less than 0.5 g for samples high in salt or fat.

6.2.3.3.3 Use an analytical portion mass of 5 g for ready to drink beverages and liquids. An analytical portion of 5 g should not be exceeded even if calculations indicate that a larger portion could be taken.

6.2.3.3.4 Use 1 g reagent water for method blanks (MBK) and optional fortified method blanks (FMB).

6.2.3.3.5 Add 1 g of reagent water with dry plant and CRMs to help control exothermic reactions.

6.2.3.4 Add 5.0 mL (7.1 g) of high purity nitric acid into each vessel, washing down any material on walls. Acid should be added drop wise until it can be established that the sample will not react violently. If foaming or reaction with the acid is observed, let the vessels sit uncovered in a clean hood until reaction subsides.

If it appears that excessive foaming would result in the sample-acid mixture expanding out of the vessel, then the closed vessel system should be used for this product.

6.2.3.5 Add 1 mL high purity 30% H_2O_2 to each vessel. It may be necessary to pre-digest for more than 20 minutes before adding H_2O_2 if samples foam excessively.

6.2.3.6 After the HNO_3 and H_2O_2 additions, the analytical portion should be completely wetted and well-mixed. Soft agitation in an ultrasonic bath may be useful to assist mixing.

A small amount of reagent water may also be added to completely wet the sample and wash material from the vessel walls.

6.2.3.7 Fill reaction chamber Polytetrafluroethylene (PFTE) liner with manufacturer's recommended base after each digestion cycle.

6.2.3.8 Cap each vessel and place vessels into vessel rack.

6.2.3.9 Place vessel rack into microwave, turn on chiller, close reaction chamber, pressurize chamber with 40 bar N_2 or Ar and begin microwave program (Table 4).

Note: Quartz or TFM vessels are recommended. Ensure that samples are completely wetted by the acid.

Table 4. Autoclave Style Microwave Digestion Program

Digestion Programs for Milestone UltraWAVE™ with 15-Position rack with Ramp to Temperature Feature						
1	00:30:00	Ramp	250°C	60°C	160 bar	1500 W
2	00:15:00	Hold	250°C	60° C	160 bar	1500 W

6.2.3.10 After the digestion finishes, allow chamber temperature (T1) to cool to 60 °C and release pressure no faster than 6 bar/min. Effervescence and vessel cap displacement may occur at higher pressure release rates resulting in sample loss.

6.2.3.11 Move cooled and depressurized vessels to an exhausting clean hood. Quantitatively transfer each digest to a clean container and dilute digestion solution to approximately 25 g with reagent water followed by 0.25 mL (0.6 g) high purity HCl (or 2.5 mL of 10% HCl solution). Dilute with reagent water to approximately 50 g. Record weight of final analytical solution to nearest 0.01 g.

6.2.3.12 Assuming 50% oxidative acid consumption, the final matrix composition is 5% HNO_3 and 0.5% HCl.

7.0 Method Quality Control

7.1 For each batch of samples processed, a method blank should be carried throughout the entire sample preparation and analytical process. These blanks will be useful in determining if samples are being contaminated. It is recommended that more than one blank be ran with each production batch.

7.2 Spiked duplicate samples should be processed on a routine basis and whenever a new sample matrix is being analyzed. Spiked duplicate samples will be used to determine precision and bias. The criteria of the determinative method will dictate frequency, but 5% (one per batch) is recommended or whenever a new sample matrix is being analyzed.

Failure of any of the QC elements described below to meet performance criteria requires reanalysis of samples analyzed prior to the loss of method control measures. A single element's QC failure does not automatically fail other elements. For example, if Zn QC fails but As QC passes, it is acceptable to report as results. If QC fails, consider whether high calories (e.g., fat/oil) may be an issue requiring test portion mass to be lowered or additional analytical solution dilutions.

7.3 The following items are common causes of erroneous results and the associated QC items follow:

7.3.1 Vessel **contamination** may be identified when analyzing duplicate analytical portions. When relative percent difference (RPD) of two duplicate analytical portions exceeds 20% and concentrations > LOQ, it is possible that one of the analytical portions has been contaminated. Analyzing duplicate portions will not indicate if the bulk composite is contaminated.

7.3.2 Inadequate **spectral interference** mitigation is identified through measurement of multiple isotopes. Where possible, two isotopes should be measured. Elemental concentrations calculated from each isotope should agree to within 20% RPD when concentrations > LOQ. If they do not, this suggests a spectral interference at one of the isotope m/z and should be further investigated and remedied.

7.3.3 **Matrix effects** may cause unwanted enhancement or depression of sample signal. Internal standards have been chosen to compensate for matrix effects but are not 100% assured. Fortified analytical portion and fortified analytical solution QC failure can indicate a matrix effect. FAPs and FASs do not correct for or indicate spectral interferences (or indicate a lack thereof).

7.4 The following is the minimum number of quality control samples analyzed with each analytical sequence:

7.4.1 Two method blanks (MBKs)

7.4.1.1 Minimum of 2 MBKs and concentration of both MBKs are ≤ MBK_C.

7.4.1.2 If 3 or more MBKs are analyzed, then at least two-thirds of MBKs are ≤ MBK_C.

7.4.1.3 MBKs exceeding MBK_C should be uncommon, i.e., 5% frequency of MBKs analyzed.

7.4.1.4 If a failure occurs due to contamination, the source of contamination should be investigated and remedied. One of the most common contamination issues is spot (microwave vessel) contamination. When spot contamination is suspected, wash vessels and run a digestion with vessels as MBKs. Place vessels back in the same rotor position (or label vessels to keep track). Analyze MBKs and find possible contaminated vessels. Repeat and/or remove contaminated vessels from use.

7.4.1.5 If a failure occurs due to polyatomic interference, increase helium flowrate and/or energy discrimination and reanalyze the entire sequence. If a failure is still present, vessels should be thoroughly cleaned, and new analytical portions must be digested.

7.4.2 Stability Check

7.4.2.1 Demonstrate instrument stability by analyzing a midrange multi-element standard containing the analytes (*e.g.,* CCV). Relative standard deviation (RSD) of ion signals must be ≤10%. If RSD > 10%, determine and correct problem before standardization. Stability problems are usually related to sample introduction.

7.4.3 Certified reference material (CRM)

Match reference material matrix as closely as possible to the cannabis product matrix. In-house RMs are acceptable if no CRM is available and/or the in-house RM is well characterized.

7.4.3.1 RM % true value recovery: 80 – 120% when concentrations > LOQ or within concentration uncertainty (converted to percent relative uncertainty) supplied on certificate, whichever is greater.

7.4.3.2 If acceptable values are not obtained, the analytical solution may be reanalyzed once. If acceptability is still not met, recalibrate and reanalyze the entire analytical sequence and/or prepare and digest new analytical portions.

7.4.4 Duplicate analytical portions are required for each product sample.

7.4.4.1 RPD < 20% for replicate analytical portions when concentration > LOQ.

7.4.4.2 If RPD < 20% is not achieved, reanalyze replicate analytical solutions once. If acceptable RPD is still not achieved, the source of imprecision should be investigated and remedied. The entire analytical sequence may need to be reanalyzed and/or new analytical portions be digested.

7.4.5 Fortified analytical portion (FAP) per sample type.

7.4.5.1 FAP % recovery can fail due to inappropriate fortification levels. We recommend analyzing the product once as a 'test' sample and then fortifying at the proper levels in a subsequent digestion (report only the second analysis result) or b) fortify duplicate portions at 'low' and 'high' levels e.g., fortify one portion at ~100 ng/g and a separate analytical portion at ~500 ng/g.

7.4.5.2 FAP preparation: It is recommended that the concentration added by fortification into the digestion vessel with the analytical portion is at the level of interest, or 50-300% of the native elemental concentration, whichever is greater. If a level of interest is not defined, it is recommended to spike at a low level standard analytical solution concentration – (i.e., using Table 2 as an example, spike at level 3 calibrant concentration).

7.4.5.3 FAP % marginal recovery: 80 – 120%.

7.4.5.4 If acceptable recovery is not obtained, ensure spike level is appropriate and reanalyze analytical solution once. If FAP fails again, reanalyze samples that followed the last acceptable FAP. If FAP fails again, prepare and digest new analytical portions.

7.4.6 Fortified analytical solution (FAS) per sample type.

7.4.6.1 FAS preparation: Spike 50-300% of the analytical solution concentration. If the native concentration range is unknown, spike at a low level standard analytical solution concentration – (i.e., using Table 2 as an example, spike at level 3 calibrant concentration). If FAS fails due to inappropriate fortification levels, then a new FAS solution must be made.

7.4.6.2 FAS % marginal recovery: 90 – 110%.

7.4.6.3 If acceptable recovery is not obtained, ensure spike level is appropriate and reanalyze analytical solution once. If FAS fails again, reanalyze samples that followed the last acceptable FAS. If FAS fails again, prepare a new FAS solution.

7.4.7 Optional fortified method blank (FMB)

7.4.7.1 FMB preparation: Spike approximately 2x LOQ – 8x LOQ.

7.4.7.2 FMB % marginal recovery: 90 – 110%.

8.0 References

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